GVH/GVL

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Incidence, Risk Factors, and Prognosis of Late Immune-Mediated Disorders after Allogeneic Hematopoietic Cell Transplantation (HCT)

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Background: Chronic graft-versus-host disease (cGVHD) remains a major barrier to successful use of HCT. The incidence, clinical presentation, course, and outcomes of GVHD-related syndromes are not well described in a multi-center setting.

Methods: The Chronic GVHD Consortium enrolled allogeneic HCT recipients in a prospective, longitudinal, observational study, and followed them closely for development of four GVHD-related syndromes: Late acute GVHD, cGVHD (both defined according to NIH consensus criteria), bronchiolitis obliterans syndrome (BOS), and cutaneous sclerosis (CS). Participants were enrolled up to 121 days after HCT as long as they had not already developed a late immune-mediated disorder. Research samples were drawn at day 100 and again at day 180 or 365 post HCT. Subjects who developed GVHD syndromes underwent additional sampling and data collection.

Results: Allogeneic HCT recipients (N=913) were enrolled at 13 centers from March 2011 to May 2014. The cumulative incidence of cGVHD was 51% with a median onset of 7.3 months (Table), and 86% of cGVHD cases were diagnosed within the first year after HCT. Late acute GVHD and BOS had particularly poor survival with 29% and 36% non-relapse mortality (NRM) at 2 years after syndrome onset. In multivariable analysis, KPS < 80% at HCT (HR: 2.6, 95% CI:1.6-4.0, p<0.001) was associated with a higher risk of late acute GVHD. Unrelated cord blood transplantation (UCBT) was associated with a lower risk of cGVHD (HR 0.5, 95% CI 0.3-0.7, p<0.001). At 2 years after HCT the probability of GVHD-free, relapse-free survival was 21% (95% CI: 17%-24%) including only the late immune-mediated disorders, and 9% (95% CI: 7%-12%) if classic acute GVHD was included as an event.

Conclusions: This is the first prospective study evaluating incidence and outcomes of GVHD-related syndromes. The onset of the syndromes varied from a median of 5.2 to 13.5 months after HCT, indicating need for continuous close monitoring and management. Amongst the GVHD-related syndromes, late acute GVHD and BOS have particularly poor overall survival, indicating a need for more effective treatments. Since research samples were banked, further studies examining biologic correlates of these syndromes are planned. Investigators interested in access to data or samples should contact the Chronic GVHD Consortium. (http://www.rarediseasesnetwork.org/cgvhd/about/ OR chronicgvhdstudies@fhcrc.org).

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Respiratory Virus (RV) from Broncho Alveolar Lavage (BAL) Prior to Hematopoietic Cell Transplantation (HCT): A Strong Predictor for Allo-Immune Mediated Lung Syndromes (allo-LS)

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Background: Allo-LS is an important complication of HCT. Early RV infection is assumed to be a predictor for allo-LS. We routinely screen patients pre-HCT for RV, both in Nasopharyngeal Aspirate (NPA) and BAL. This study evaluates the impact of RV RNA-DNA-positivity pre-HCT from NPA or BAL respectively on the occurrence of allo-LS.

Methods: We prospectively included all consecutive pediatric HCT recipients from January 2007– October 2013. A PCR on a panel of RV (Adenovirus, Coronavirus, Rhinovirus, RSV, Bocavirus, Influenzavirus A and B, Parainfluenza 1-3 and 2-4, human-Metapneumovirus) was performed on BAL and NPA samples a week prior to HCT. Primary endpoint was allo-LS, defined according to international criteria as Idiopathic Pneumonia Syndrome (acute respiratory symptoms, pulmonary infiltrates, absence of infection) or Bronchiolitis Obliterans (HRCT changes such as air trapping and ground glass, abnormal obstructive pulmonary function test, absence of infection). Cox proportional hazard models were used for analyses.

Table

Syndrome	Number of cases	Cumulative incidence at 2 years (95% CI)	Median time to onset, mos (range)	NRM 2 years after onset (95% CI)	Survival 2 years after onset (95% CI)
Late acute GVHD	85	11% (9%-14%)	5.2 (3.2-15.6)	29% (15%-54%)	57% (33%-76%)
Chronic GVHD	339	51% (47%-55%)	7.3 (1.8-22.0)	17% (11%-26%)	77% (67%-84%)
BOS	25	5% (3%-8%)	11.4 (2.8-21.7)	36% (15%-90%)	62% (22%-86%)
CS	44	10% (7%-13%)	13.5 (4.0-26.5)	12% (4%-36%)	88% (65%-96%)

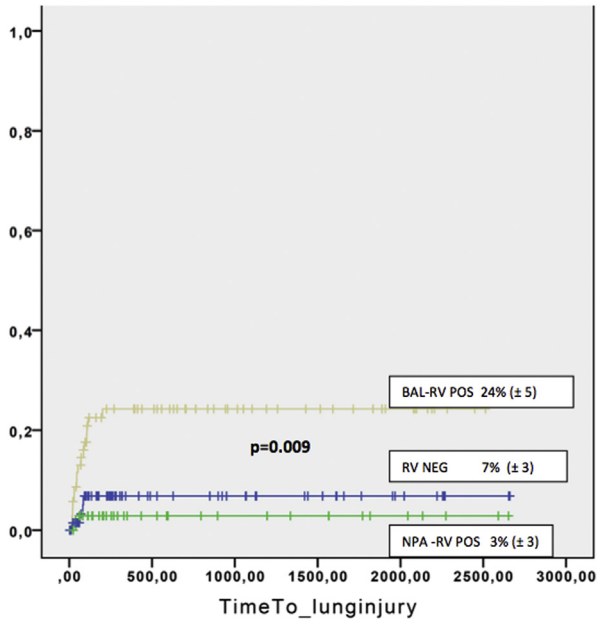

Figure 1. Cumulative incidence of allo-LS for BAL-RV positive, NPA-RV positive and RV negative patients

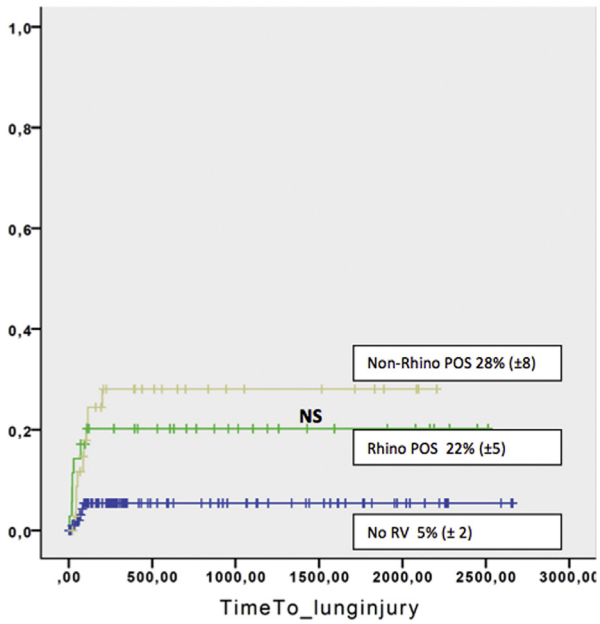

Figure 2. Cumulative incidence of allo-LS for Rhinovirus positive, non-Rhinovirus positive and RV negative patients.

Results: 178 patients were included, median age 6.8 yr (0.6-22.7), transplanted for leukemia/lymphoma (51%), bone marrow failure syndromes (9%), primary immune deficiency (PID) (18%) and non-PID benign disorders (22%). Myeloablative conditioning regimen was used; chemotherapy based in 85%, containing Total Body Irradiation in 15%. Donors used: Matched family (26%), unrelated cord blood (54%) and unrelated bone marrow (20%). In 57% of patients pre-HCT NPA was positive for RV. RV was detected in 40% of BAL samples. In 37% of NPA-RV positive patients BAL was negative, whereas only 7% of BAL positives were NPA negative. Rhinovirus was the most frequently detected virus (43%). There was a similar distribution of the various viruses in BAL and NPA. Most patients had no or very mild signs of upper respiratory tract infection at time of sampling. In multivariate analyses BAL RV positivity was the only predictor associated with allo-LS: HR 3.89, 95% CI 1.41–10.40; p=0.009). NPA-only positivity was not associated with allo-LS (Fig. 1). No significant difference was found between Rhino- and nonRhino virus in the risk for allo-LS (Fig. 2).

Conclusion: RV positivity is frequently seen in pediatric patients pre-HCT. Rhinovirus was the most commonly found RV. RV positivity in BAL pre-HCT is a strong predictor for allo-LS in children post-HCT. This association was similar for Rhino- and nonRhinovirus positive patients. These data support the importance of identifying patients at risk for allo-LS by pre-HCT BAL. Early recognition may guide preventive strategies: e.g. postponement of elective HCTs and delayed tapering of immune-suppression.

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Prevention of Acute GVHD by Ex Vivo Expanded Umbilical Cord Blood Derived Regulatory T Cells (Treg)

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We previously showed that adoptive transfer of 3 million Treg per kilogram recipient body weight could reduce the risk of acute GVHD (aGVHD) (39% vs. 61%, *p*-0.05; Blood 117:1061) in recipients of double UCBT. We hypothesized that control of GVHD was suboptimal, likely related to the low ratio of Treg to T cells in the UCB graft (i.e., 0.3:1.0). Our group previously demonstrated optimal murine GVHD control with Treg:Teffector ratio $\geq$ 1:1. A genetically modified K562 cell line, termed KT64/86, that expressed CD64, a high affinity Fc receptor loaded with anti-CD3 antibody, and CD86, the natural ligand of CD28/CTLA-4, was used to manufacture higher doses of Treg from a single UCB unit, that retained the CD4+CD25+FoxP3+ CD127- phenotype and had potent in vitro and in vivo (murine xenogeneic) suppression of T cell proliferation. Using this methodology, we performed a phase I dose escalation trial starting with 3 million donor Treg/kg. Patients who were candidates for nonmyeloablative double UCBT were eligible. Ten patients received Cy50/FLU200/TBI200 nonmyeloablative conditioning with sirolimus and mycophenolate mofetil immunosuppression. Thawed Tregs underwent a single CD25 positive selection and were cultured for 18 $\pm$1 days in the presence of KT64/86 (1:1) supplemented with 300 units/mL rhIL-2; cultures were restimulated with KT64/86 on day 12. In the dose escalation, 3 to 100 million Tregs/kg were infused on day +1 and patients monitored for infusional toxicity and GVHD. Fifteen concurrent patients received same treatment regimen but no Treg served as controls for clinical endpoints.